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Efficacy and Phytotoxicity of Fungicides for Control of Botrytis Gray Mould on Container-Grown Conifer Seedlings

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Efficacy and Phytotoxicity of Fungicides for Control of Botrytis Gray Mould on Container-Grown Conifer Seedlings

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ABSTRACT

The fungicides anilazine, captan, dichloran, folpet, iprodione, and thiram were tested for efficacy against gray mould, *Botrytis cinerea* (Fries) Persoon, on container-grown seedlings of white spruce, western redcedar, western hemlock, Sitka spruce, and Douglas-fir. Phytotoxicity of the fungicides to the seedlings was also determined. Beginning in the 14th week after germinant emergence, the fungicides were applied bi-weekly for the next 2 months. To ensure that adequate inoculum of the pathogen was present, the seedlings were inoculated with a mixture of 10 isolates of *B. cinerea* after the first fungicide application, and on alternate weeks thereafter for a total of six inoculations. Growth of all species except Douglas-fir was inhibited by one or more of the fungicides. Iprodione gave excellent *Botrytis* control on all seedling species. Anilazine gave adequate control on all species except Douglas-fir and western redcedar, and some needle browning was observed on both spruce species. Gray mould control on western redcedar was excellent with thiram, and good on all other species except western hemlock. Folpet gave good control of *Botrytis* on Douglas-fir and Sitka spruce. Disease control was excellent with dichloran on western hemlock and white spruce. Captan provided good gray mould control on Douglas-fir. *In vitro* tolerance tests showed that all 10 of the *B. cinerea* isolates used in the experiment were sensitive to iprodione and dichloran, and to captan at the 2 X dosage. All isolates were tolerant to anilazine; most were tolerant to thiram and folpet, and to captan at the 0.5 X and 1 X dosage.

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INTRODUCTION

In British Columbia, gray mould caused by the fungus *Botrytis cinerea* is a major disease on container-grown conifer seedlings, especially in coastal, mainland and Vancouver Island nurseries. While the disease is particularly severe in western hemlock (*Tsuga heterophylla* (Raf.) Sarg.) and Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), it can also affect most tree species grown for reforestation (James 1984). The high humidity and moderate temperatures of container nurseries all favour the development of gray mould (Sutherland and Van Eerden 1980; James 1984).

Gray mould management currently entails the use of cultural practices to alter the nursery environment so that it is not conducive to the disease, and the use of fungicides as needed. Examples of effective cultural practices include increasing ventilation and reducing the length of time that seedling foliage is wet. At present, the main fungicides used in British Columbia for *Botrytis* control are benomyl, captan, and chlorothalonil (B.C. Ministry of Agriculture and Food 1985). In the United States, several other fungicides are used, such as iprodione, dichloran, and thiram (James 1984).

Studies on agriculture and forestry crops in the United States, New Zealand, and Canada have shown that already *B. cinerea* tolerance has developed to currently used fungicides. *Botrytis* tolerance occurs with the continued, long-term use of one or two fungicides (James and Gilligan 1983). Barak and Edgington (1984) found tolerance to folpet, thiram, and iprodione among several *B. cinerea* isolates, and tolerance to iprodione and dichloran was recorded for several *B. cinerea* isolates from vegetable crops (Beever and Brien 1983). *Botrytis* isolates from conifers, were found to be tolerant to benomyl, chlorothalonil, and captan, but little tolerance existed to iprodione or dichloran (Gillman and James 1978; Cooley 1981; James and Gilligan 1983). A recent survey conducted for the B.C. Ministry of Forests and Lands found that 74% of the *Botrytis* isolates from ministry and private nurseries were tolerant to benomyl and 60% were tolerant to chlorothalonil¹. The development of fungicide-tolerant strains of *Botrytis*, particularly to benomyl, chlorothalonil, and captan, emphasized the need for a wider variety of fungicides for use against *Botrytis* in British Columbia. The objective of the present experiments was to evaluate (1) the efficacy of six fungicides against *Botrytis* and (2) the possible phytotoxicity of the fungicides to tree seedlings. All of these fungicides are currently registered for use on agricultural crops in Canada. *In vitro* tests were also conducted to determine if the isolates of *B. cinerea* used in the research were tolerant to the six fungicides. The results of the experiments should be of value for obtaining minor use registration of the fungicides in British Columbia forest nurseries.

¹ Glover, M.M. 1985. A survey of fungicidal tolerance of *Botrytis cinerea* in British Columbia Container nurseries. B.C. Min. For. Lands Unpublished report.

MATERIALS AND METHODS

Fungicides, Tree Species, and Experimental Design

The six fungicides (with trade names in parentheses) evaluated for *B. cinerea* control were: dichloran (Botran), iprodione (Rovral®), thiram (Thiram®), anilazine (Dyrene®), folpet (Phaltan®), and captan (Captan®). The fungicides were tested at multiples of 1, 2, and 0.5 X the dosage rates with the 1 X rate being 0.94, 1.2, 3.75, 2, and 2.04 g/L of product, respectively². The different rates were used to determine phytotoxicity thresholds for each fungicide. The tree species used (Ministry of Forests and Lands seed lot number in parentheses) were white spruce (*Picea glauca* (Moench) Voss), (4177); Sitka spruce (*P. sitchensis* (Bong.) Carr.) (4184); coastal Douglas-fir (7752); western hemlock (3021); and western redcedar (*Thuja plicata* Donn.) (3546).

Seven treatments were tested (six fungicides and controls) at three dosage levels. Controls received water instead of fungicide applications, with the three dosage levels consisting of inoculation with *Botrytis* (control inoculation), and two control treatments with no *Botrytis* (designated control NB5 and NB14). Each of the five tree species was replicated four times, with a single 211-styroblock containing one tree species, i.e., it was one replication. To facilitate the fungicide application, the 20 blocks for each fungicide dosage (five species and four replications) were randomly grouped together; otherwise, the 21 treatment sections (seven treatments X three dosages) were randomized throughout the greenhouse.

Seedling cultural procedures

Seedlings were grown in styroblocks containing a 3:1 peat:vermiculite growing medium with 3 kg/m³ dolomite lime and 6.5 kg/m³ 18-6-12 Osmocote slow release fertilizer. Seeds of all the tree species were double sown to ensure one germinant per cavity, then covered with forestry sand. Seeding was done on April 16 and 17, 1985. The styroblocks were misted with water by an overhead boom until the growing medium was saturated, and then they were irrigated three times a day. Misting was increased after germination to five times a day, with additional watering as needed on sunny days. The seedlings were thinned (May 6-17 except for western hemlock, which was thinned from May 22 - 24) to one per cavity, and the misting nozzles were replaced with regular irrigation nozzles.

To prevent excessive height growth, Douglas-fir seedlings were drought stressed from September 1 to the end of the experiment (i.e., no water was applied until the seedlings reached the wilting point). Watering coincided with fungus or fungicide applications to saturate the seedlings before applications.

The seedlings were fertilized with 10-52-17 (Green Valley) at 750 g/1000 L from May 27 to June 6, and from August 4 to the end of the experiment. From June 7 to August 4, 20-20-20 (Peters) at 625 g/1000 L was used. The fertilizer was applied through the overhead boom at each watering, using a Gewa® injector system.

After germinant emergence, sodium vapour lights were used to extend the photoperiod to 18 h/day (400 lux) and prevent seedling bud set. Throughout the growing season a shade cloth shaded the seedlings to enhance seedling growth and render them more susceptible to *Botrytis*. Beginning on August 1, shading was gradually reduced to harden off the seedlings. The greenhouse temperature was kept at 22°C during germination, but after thinning the temperature was reduced to 15°C at

² Because no previous tests have been made with these fungicides against *B. cinerea* on tree seedlings in Canada, the 1 X dosage rate for each material that was selected for testing is an existing label rate for *B. cinerea* control on other crops. These rates were the ones recommended by the B.C. Ministry of Agriculture and Food (BCMAF), or other sources, for the six fungicides on other crops, i.e., the dichloran rate was the one recommended for roses and chrysanthemums (BCMAF 1984); the iprodione rate was recommended for general use (BCMAF 1984); the thiram rate was recommended for general use (BCMAF 1985); the anilazine rate was recommended for use on strawberries; the folpet rate was recommended for use against powdery mildews (BCMAF 1985); the captan rate was the same as recommended for damping-off (BCMAF 1985). None of these fungicides or rates should be used on conifer seedlings until the fungicides are officially registered for use.

night. The 22/15°C thermoperiod was continued until October 9, when a gradual reduction to 10°C days and 5°C nights began. The length of the thermoperiod was adjusted seasonally to coincide with the natural daylength.

Fungicide evaluation

All fungicides were wettable powders and the carrier was tap water. After the three control treatments were sprayed with tap water, the fungicides and the controls were applied with 5-L Gloria hand sprayers at an application pressure of approximately 3 bars, maintained until the sprayer was empty. A separate sprayer was used for each fungicide and control. For each fungicide the 0.5 X dosage was applied first, followed by the 1 X and 2 X dosages. Sprayers were calibrated before the first application to determine the amount of liquid needed for complete coverage of the seedlings. For the first three times the fungicides were applied, 5 L of fungicide mixture was used for each treatment (20 styroblocks). As the seedlings grew, this was increased to 6 L per treatment for the remaining seven applications. To prevent fungicide drift and cross-contamination of the seedlings, plastic sheets were set up between adjacent treatments on the same bench. Fungicides were first applied on July 25 and then every other week until October 31, for a total of eight applications.

Botrytis inoculum

Ten isolates of *B. cinerea* (Glover 1985),³ originally isolated from several conifer seedling species, were used (Appendix 1). Preliminary experiments revealed that some of the isolates were tolerant to captan, benomyl, and chlorothalonil fungicides. All isolates were maintained on potato dextrose agar (PDA) slants at 5°C. Fresh slants made from the original isolates in April and May were used to conduct *in vitro* tests for tolerance to the six fungicides and to inoculate the PDA for growing the *Botrytis* inoculum. To obtain the inoculum, three petri plates were inoculated with mycelium from fresh culture slants 2 weeks before each fungus inoculation. After the *Botrytis* sporulated, the plates were flooded with sterile distilled water and the mycelium was scraped to loosen the spores. The liquid was strained through two layers of cheesecloth and put into a spray bottle. One autoclavable tub containing 750 ml PDA was inoculated with the liquid from each isolate. The tubs were then placed in autoclavable bags, closed and incubated in the greenhouse for one week. On the day of fungus inoculation, 1 L of tap water was added to each tub and the mycelium was scraped. The liquid was poured through two layers of cheesecloth and the spores of all isolates were combined. The isolates were mixed to ensure a uniform inoculum. The spore count for each inoculation average 1.99×10^6 spores per millilitre. A Gloria sprayer was used to inoculate the seedlings, with 12 L inoculum being used for each *Botrytis* application. Inoculations were done on August 1, 15, and 29, September 27, and October 10 and 23. The inoculations were alternated weekly with the fungicide spraying, but the September 12 and November 7 inoculations were missed because two sets of isolates did not grow.

Assessment of efficacy

Five weeks after the final fungus inoculation and fungicide treatments, each block was assessed according to two rating systems. An efficacy rating of 1 to 4 was obtained by estimating the percentage of seedlings in each block affected by *Botrytis* (i.e., 1 = 0 - 25% of the seedlings affected and 4 = 76 - 100%). The severity of the disease on the seedlings in each block was determined on a scale of 1 to 6: 1 = very light (a few bottom needles affected), 2 = light (lower needles affected), 3 = light moderate (lower 1/4 of seedling affected), 4 = moderate (lower 1/3 of seedling affected), 5 = moderate severe (lower 2/3 of seedling affected), and 6 = severe (entire seedling affected or dead). Also noted was the location of the disease and whether the pathogen was actively sporulating (presence of conidia).

For each seedling species these ratings were then ranked from 1 to 20 (6 fungicides X 3 dosages + 2 controls): The lower the rank, the better the fungicide. The ranks were analyzed using the Kruskal-Wallis non-parametric analysis of variance (Steel and Torrie 1980). Significant differences between

³Glover, 1985

the ranks were identified by a pairwise comparison test (Hettmansperger 1984). The probability of a type 1 error among all comparisons was 10%; the probability of a type 1 error in an individual comparison was 0.05%.

Phytotoxicity of the fungicides

Beginning in mid-December, 20 randomly selected seedlings from each styroblock were sampled for phytotoxic effects. The seedlings selected were not always free from *Botrytis* and therefore any supposedly phytotoxic effects noted may have been a result of the disease. This is also true of the uninoculated controls, which were heavily infected with *Botrytis*. The seedlings were placed in labelled plastic bags and stored up to one month in waxed cardboard boxes at 0°C until the height (from root collar to bud tip) and stem diameter (at root collar) of each seedling was measured.

The data were analyzed for homogeneous variances using Bartlett's test (Walpole 1974). A one-way analysis of variance (ANOVA) was conducted on the average heights and stem diameters for each tree species and fungicide. Included in this analysis was the mean of the uninoculated controls as the 0 X dosage of each fungicide. Duncan's multiple-range test was used to determine the significance of dosage differences at the 5% probability level.

In Vitro Tolerance Test

The *in vitro* tests to determine *B. cinerea* resistance to each fungicide were conducted for each *Botrytis* isolate used in the experiment (Appendix 1). The methods were the same as those used in the earlier survey of *Botrytis* tolerance (Glover 1985).⁴ Petri plates containing PDA were inoculated with mycelium from the slants made from the original *Botrytis* isolates. Once the cultures had sporulated, spores were transferred to fungicide-amended PDA plates. To make the amended medium, PDA was autoclaved and cooled to 50°C, fungicide was added, and the medium was stirred and the plates poured. The resulting medium contained fungicide concentrations that were the same as applied to the seedlings.

Each isolate was tested with each fungicide at the three dosages and on unamended PDA (control) for a total of 21 plates per isolate. There were four replications on each plate. Whenever possible, each replication of the different treatments was taken from the same area of the isolate plate. Spores from the isolate plates were transferred to a spot on the control and amended plates, using a sterile inoculating loop and sterile distilled water as the carrier. After inoculation, the plates were stored in plastic bags at room temperature (20°C) under light. For assessment, each replicate per plate was rated based on evidence of *Botrytis* growth, but no distinction was made regarding amount of growth. An isolate was deemed tolerant if at least two of the four replicates showed growth at 3 days, or if three or more showed growth after 7 days.

RESULTS⁵

Fungicide Efficacy and Phytotoxicity

The Kruskal-Wallis non-parametric analysis of variances showed that there was no significant difference between fungicide dosages. Dosage as a factor was thus ignored, and the ranks within each species were totalled for each fungicide. These totals were used to assign new ranks (overall rank, Appendices 2 and 3) from 1 to 7 (6 fungicides + 1 control). In all cases the controls were more severely diseased than the treated seedlings. To facilitate the assessment of the fungicides, the overall efficacy and severity ranks for each fungicide were summed and used to create a composite rank from 1 to 6 (6 fungicides) within each species (Table 1). For each fungicide, the individual species ranks were then totalled and used to assign an overall composite rank (Table 1).

⁴Ibid

⁵The data reported here are mainly summaries of data from: M.M. Glover. 1986. Assessment of phytotoxicity and efficacy of fungicides for control of *Botrytis* gray mould on seedlings of white spruce and other conifers. Canada-B.C. Forest Resource Development Agreement, Victoria, B.C. Unpublished report.

TABLE 1. Composite fungicide efficacy and severity rankings^a

Treatment	Douglas-fir	Western hemlock	Sitka spruce	White spruce	Western redcedar	Overall composite rank
Anilazine	6	3	2	4	4	4
Captan	3	6	6	6	5	6
Dichloran	5	2	4	5	6	5
Folpet	4	5	3	2	3	3
Iprodione	1	1	1	1	1	1
Thiram	2	4	5	3	2	2

^a The lower the ranking, the better the control of *Botrytis*. See Appendices 2 and 3 for the data on which the above table is based.

Before *Botrytis* inoculation, a natural outbreak of the disease affected inoculated and uninoculated seedlings in all control blocks, particularly in the western hemlock controls. Compared to the control, all fungicides reduced gray mould occurrence. Appendices 2 and 3 give the rankings for efficacy and severity for each fungicide and dosage by species. Table 1 shows the composite efficacy and severity ranks for each fungicide and species and the overall composite rank for each fungicide. Because of an oversight, six replicates in the thiram 1 X treatment were missed, but provision was made in the analysis of the phytotoxicity data for the unequal number of replications. Mean shoot heights and stem diameters for the seedlings in each treatment are given in Tables 2 and 3. The following summarizes in order of efficacy (ability to control gray mould) and phytotoxicity, if any, the results for each fungicide (Table 1).

Iprodione (overall composite ranking = 1):

EFFICACY: This fungicide provided excellent *Botrytis* control on all tree species. In western hemlock, infection was reduced from severe to light. In Douglas-fir, the best control was found at the 2 X dosage.

PHYTOTOXICITY: Iprodione inhibited growth of all species except Douglas-fir. Heights and stem diameters of western hemlock were significantly less than the uninoculated controls at the 2 X fungicide dosage, as were heights of Sitka spruce, white spruce, and western redcedar.

Thiram (overall composite ranking = 2):

EFFICACY: Thiram gave excellent control of *Botrytis* on western redcedar and Douglas-fir, and good control on the remaining tree species.

PHYTOTOXICITY: Heights of Sitka spruce at all dosage levels were significantly less than the uninoculated controls, as were the heights and stem diameters of western hemlock at one or more of the fungicide dosages.

Folpet (overall composite ranking = 3):

EFFICACY: Folpet gave good control of *Botrytis* in white spruce and Sitka spruce; however, control was adequate in western redcedar and Douglas-fir and very poor in western hemlock.

PHYTOTOXICITY: Folpet adversely affected growth of all species except Douglas-fir. Heights and stem diameters of Sitka spruce and western hemlock were significantly less than the uninoculated controls at one or more of the fungicide dosages, as were heights of white spruce and western redcedar.

TABLE 2. Mean height (cm) at end of growing season after fungicide treatment

Treatment dosage	Mean height (cm) by tree species ^a				
	Douglas-fir	Western hemlock	Sitka spruce	White spruce	Western redcedar
Control ^b	29.9	22.6	29.3	21.6	33.8
Anilazine					
0.5 X	29.0a	20.2a	25.7*	19.5a	32.1a
1X	31.9*	19.9a	24.3*	20.4a	31.3a
2X	29.8a	21.0a	26.4*	20.7a	29.8*a
Captan					
0.5 X	28.2b	22.2b	26.4*	21.3b	31.7b
1X	28.2b	22.1b	27.1*a	21.1b	28.4*
2X	30.0b	21.2b	28.3a	20.1b	32.7b
Dichloran					
0.5 X	29.6c	17.4*	25.5*	19.1c	30.7*
1X	30.2c	19.3*	26.2*	21.0c	31.1*
2X	29.5c	19.5*	27.2*	21.4c	33.9c
Folpet					
0.5 X	28.4d	20.3c	26.2*	18.0*	30.2*d
1X	29.4d	21.0c	25.0*	20.9d	28.5*
2X	28.2d	19.1c	28.7b	21.6d	32.2d
Iprodione					
0.5 X	31.1e	18.0*	27.2*	19.1*	31.4e
1X	29.2e	18.4*	26.2*	20.6*	30.4*e
2X	29.2e	17.7*	26.6*	18.5e	28.9*e
Thiram					
0.5 X	29.4f	19.8d	27.4*	20.0f	32.0f
1X	29.8f	17.9*	26.8*	18.6f	30.5f
2X	30.1f	16.9*	26.2*	20.0f	32.5f

^a Within each species and fungicide, heights followed by the same letter are not significantly different ($\alpha = 0.05$) using Duncan's multiple range test. Any heights marked with an "*" are significantly different from the control for that species. See "Discussion and Conclusions" for a statement on the practical effects of any reductions in seedling heights.

^b Controls were uninoculated and treated with tap water. Inoculated controls were not used in this analysis because they were damaged by disease. Healthy seedlings were selected for each treatment.

Anilazine (overall composite ranking = 4):

EFFICACY: Anilazine provided adequate *Botrytis* control on all tree species except Douglas-fir, on which it was slightly less effective. Gray mould control was particularly effective in western hemlock and Sitka spruce.

PHYTOTOXICITY: Heights and stem diameters of Sitka spruce and western redcedar were significantly less than in the uninoculated controls, whereas heights of Douglas-fir in the 1 X dosage were significantly greater than in the uninoculated controls. After the first application of the fungicide, a light browning of the tips of the lower needles was noted at all dosages on both white spruce and Sitka spruce, but subsequent fungicide applications did not worsen this condition.

Dichloran (overall composite ranking = 5):

EFFICACY: Dichloran provided excellent gray mould control on western hemlock, reducing severity of infection from severe to light. Very good control was noted in Sitka spruce, while adequate control was observed in the remaining species.

PHYTOTOXICITY: Dichloran inhibited growth in western hemlock, Sitka spruce, and western redcedar at one or more of the fungicide dosages. Heights and stem diameters of western hemlock were significantly less than in the uninoculated controls, as were the heights of Sitka spruce and western redcedar.

Captan (overall composite ranking = 6):

EFFICACY: Captan reduced *Botrytis* on all tree species. Compared to the control, captan was not as effective against *Botrytis* as the other fungicides. Control was poor on western hemlock, which sustained moderately severe damage but control was good on Douglas-fir and western redcedar.

PHYTOTOXICITY: Height growth of Sitka spruce and western redcedar was inhibited at one or more of the fungicide dosages, as was the stem diameter of western redcedar. Stem diameters of white spruce were significantly greater than the uninoculated controls.

TABLE 3. Mean stem diameter (cm) at end of growing season after fungicide treatment.

Treatment dosage	Mean stem diameter (cm) by tree species ^a				
	Douglas-fir	Western hemlock	Sitka spruce	White spruce	Western redcedar
Control ^b	0.224	0.206	0.218	0.189	0.186
Anilazine					
0.5 X	0.222a	0.191a	0.204a	0.186a	0.177a
1X	0.218a	0.200a	0.193*a	0.197a	0.192b
2X	0.207a	0.190a	0.205a	0.201a	0.171*a
Captan					
0.5 X	0.224b	0.202bc	0.223b	0.203*	0.192c
1X	0.231b	0.213b	0.227b	0.204*	0.193c
2X	0.227b	0.185*c	0.206b	0.182b	0.185c
Dichloran					
0.5 X	0.235c	0.172*	0.214c	0.193c	0.181d
1X	0.235c	0.183*	0.200c	0.187c	0.186d
2X	0.223c	0.196*	0.213c	0.93c	0.185d
Folpet					
0.5 X	0.211d	0.195d	0.200*d	0.180d	0.186d
1X	0.235d	0.183*d	0.202*d	0.197d	0.174d
2X	0.220d	0.172*	0.216d	0.192d	0.186d
Iprodione					
0.5 X	0.227e	0.171*	0.224e	0.188e	0.185e
1X	0.237e	0.184*	0.213e	0.198e	0.182e
2X	0.224e	0.172*	0.204e	0.187e	0.176e
Thiram					
0.5 X	0.230f	0.192e	0.213f	0.198f	0.193f
1X	0.229f	0.175*e	0.206f	0.177f	0.182f
2X	0.236f	0.182*e	0.212f	0.195f	0.197f

^a Within each species and fungicide, stem diameters followed by the same letter are not significantly different ($\alpha = 0.05$) using Duncan's multiple range test. Any stem diameters marked with an "*" are significantly different from the control for that species. See "Discussion and Conclusions" for a statement on the practical effects of any reductions in seedling stem diameters.

^b Controls were uninoculated and treated with tap water. Inoculated controls were not used in this analysis because they were damaged by disease. Healthy seedlings were selected for each treatment.

In Vitro Tolerance Test

All the *Botrytis* isolates were tolerant to anilazine at all rates, and to thiram at the 0.5 X and 1 X dosages (Table 4). Only one isolate (B2) was sensitive to thiram at the 2 X dosage. All isolates were tolerant to folpet at 0.5 X, and seven were tolerant at 1 X and 2 X. Seven of the isolates were tolerant to captan at 0.5 X and five were tolerant at 1 X. All isolates were sensitive to captan at 2 X, and to iprodione and dichloran, regardless of the dosage.

TABLE 4. Average tolerance (T) or sensitivity (S) of *Botrytis cinerea* to the test fungicides

Fungicide	<i>Botrytis</i> isolate ^a										Average
	B1	B2	B4	B11	B24	B27	B29	B30	B31	B32	
Anilazine											
0.5 X	T	T	T	T	T	T	T	T	T	T	T
1X	T	T	T	T	T	T	T	T	T	T	T
2X	T	T	T	T	T	T	T	T	T	T	T
Captan											
0.5 X	T	S	S	S	T	T	T	T	T	T	T
1X	S	T	S	S	T	T	T	S	T	S	S
2X	S	S	S	S	S	S	S	S	S	S	S
Dichloran											
0.5 X	S	S	S	S	S	S	S	S	S	S	S
1X	S	S	S	S	S	S	S	S	S	S	S
2X	S	S	S	S	S	S	S	S	S	S	S
Folpet											
0.5 X	T	T	T	T	T	T	T	T	T	T	T
1X	S	T	T	S	T	T	T	T	T	S	T
2X	T	S	S	T	T	T	T	S	T	T	T
Iprodione											
0.5 X	S	S	S	S	S	S	S	S	S	S	S
1X	S	S	S	S	S	S	S	S	S	S	S
2X	S	S	S	S	S	S	S	S	S	S	S
Thiram											
0.5 X	T	T	T	T	T	T	T	T	T	T	T
1X	T	T	T	T	T	T	T	T	T	T	T
2X	T	S	T	T	T	T	T	T	T	T	T

^a Sources given in Appendix table 1.

DISCUSSION AND CONCLUSIONS

The objective of these experiments was to obtain data for possible label extensions of several fungicides for *Botrytis* control, and therefore the discussion considers each fungicide separately. Table 5 summarizes the results by species. In evaluating fungicides, it is important to balance phytotoxic effects with the degree of control provided, while also considering the possibility that the pathogen might develop tolerance to the fungicide. As tolerance to most of the test fungicides has been found in this or previous studies (Gillman and James 1978; Cooley 1981; Beever and Brien 1983; James and Gilligan 1983), the use of these fungicides must be rotated with other products. Studies involving some of the six test fungicides have been carried out in the United States. In three separate trials conducted on western larch (*Larix occidentalis* Nutt.) and lodgepole pine (*Pinus contorta* var *latifolia* Dougl.), iprodione and captan provided very good control of gray mould. Dichloran reduced the severity of *Botrytis* infection, particularly on lodgepole pine. In all studies, fungicides inhibited seedling height growth (James *et al.* 1982; James and Genz 1983; James and Woo 1984).

Because this experiment dealt with fungicide efficacy, special care was taken to ensure good coverage and penetration of the fungicides into the seedling canopy. These are important factors in assessing efficacy.

Questions may arise because the controls were not disease-free, but because of greenhouse space limitations it was not possible to grow the uninoculated controls separately. However, the disease in the controls probably did not affect the overall results greatly, given the very noticeable differences between treated and untreated seedlings.

Based on the results of this experiment, the following conclusions have been reached about the efficacy, phytotoxicity, and usefulness of pursuing registration of the six test fungicides for management of gray mould (Table 5). It should be noted that although height growth or stem diameter of certain species of seedlings were occasionally reduced by some of the fungicides – especially at the 2 X dosage rate – and although these reductions were sometimes statistically significant, the overall reductions in seedling growth were seldom great enough to prevent their meeting Ministry of Forests and Lands grading standards.

Iprodione (rank 1):

Although Iprodione caused some reduction in growth of western hemlock, Sitka spruce, white spruce, and western redcedar seedlings, it provided excellent disease control for all species. Growth losses would likely be compensated by the protection provided by Iprodione. The *in vitro* tests suggest there is no *Botrytis* tolerance to Iprodione; however, tolerance can develop quickly with the continuous use of a fungicide. Label extension for *Botrytis* control on western hemlock, western redcedar, Sitka spruce, white spruce, and Douglas-fir should be pursued for Iprodione.

Thiram (rank 2):

Thiram gave excellent control of *Botrytis* on western redcedar and Douglas-fir and good control on the other tree species except western hemlock and Sitka spruce. Seedling growth of western hemlock and Sitka spruce was reduced by thiram application. The *in vitro* tests strongly suggest that *Botrytis* tolerance to thiram exists, so this fungicide could be used in an infrequent rotation with other fungicides. Label extension should be pursued for the control of *Botrytis* on western redcedar, Douglas-fir, Sitka spruce, and white spruce.

Folpet (rank 3):

Folpet provided good gray mould control on Sitka spruce, white spruce, and western redcedar, and adequate control on Douglas-fir. Control was poor on western hemlock. Folpet reduced seedling growth of all species except Douglas-fir. The *in vitro* tests suggest that tolerance to folpet exists or could develop rapidly. Therefore, this fungicide should only be used in rotation with other products. Label extension of folpet for control of *Botrytis* on Sitka spruce, white spruce, western redcedar, and perhaps Douglas-fir should be pursued.

TABLE 5. Summary of results by seedling species

Douglas-fir

Fungicide	Efficacy rank	Severity rank	Effect on height	Effect on stem diameter	Composite rank	Recommend label extension
anilazine	5.5	6.0	b	-	6.0	Yes
captan	4.0	1.0	-c	-	4.0	Yes
dichloran	5.5	4.0	-	-	5.0	Perhaps
folpet	2.0	5.0	-	-	2.0	Perhaps
iprodione	1.0	3.0	-	-	1.0	Yes
thiram	3.0	2.0	-	-	3.0	Yes

Western hemlock

Fungicide	Efficacy rank	Severity rank	Effect on height	Effect on stem diameter	Composite rank	Recommend label extension
anilazine	3.0	3.0	-	-	3.0	Yes
captan	5.0	6.0	-	Dd	5.0	Yes
dichloran	2.0	2.0	D	D	2.0	Yes
folpet	6.0	4.0	-	D	6.0	No
iprodione	1.0	1.0	D	D	1.0	Yes
thiram	4.0	5.0	D	D	4.0	No

Sitka spruce

Fungicide	Efficacy rank	Severity rank	Effect on height	Effect on stem diameter	Composite rank	Recommend label extension
anilazine	3.0	2.0	D	D	2.0	No
captan	5.0	6.0	D	-	6.0	Yes
dichloran	6.0	1.0	D	-	4.0	Yes
folpet	2.0	5.0	D	D	3.0	Yes
iprodione	1.0	3.0	D	-	1.0	Yes
thiram	4.0	4.0	D	-	5.0	Yes

White spruce

Fungicide	Efficacy rank	Severity rank	Effect on height	Effect on stem diameter	Composite rank	Recommend label extension
anilazine	2.0	4.5	-	-	2.0	No
captan	6.0	6.0	-	I	6.0	Yes
dichloran	5.0	4.5	-	-	5.0	Perhaps
folpet	3.0	3.0	D	-	4.0	Yes
iprodione	1.0	1.0	D	-	1.0	Yes
thiram	4.0	2.0	-	-	3.0	Yes

TABLE 5. Summary of results by seedling species (continued)

Western redcedar

Fungicide	Efficacy rank ^a	Severity rank	Effect on height	Effect on stem diameter	Composite rank	Recommend label extension
anilazine	3.0	5.0	D	D	3.0	Yes
captan	5.0	4.0	D	-	5.0	Yes
dichloran	6.0	6.0	D	-	6.0	Yes
folpet	4.0	3.0	D	-	4.0	Yes
iprodione	1.0	2.0	D	-	1.0	Yes
thiram	2.0	1.0	-	-	2.0	Yes

^a The lower the efficacy or severity rank, the better control provided by that fungicide. Controls were always more diseased than treated seedlings.

^b I = significant increase at one or more of the fungicide dosages when compared to the inoculated controls.

^c - = no significant difference at any fungicide dosage when compared to the uninoculated controls.

^d D = significant decrease at one or more of the fungicide dosages when compared to the uninoculated controls.

Anilazine (rank 4):

Anilazine provided adequate *Botrytis* control on all tree species except Douglas-fir and western redcedar, where it was slightly less effective. Reduced seedling growth of western redcedar, Sitka spruce, and white spruce would be offset by the greater number of disease-free seedlings, but further studies may be needed to determine possible phytotoxicity of anilazine to Sitka and white spruce species, particularly on 0- to 15-week-old seedlings. The *in vitro* tests demonstrated that tolerance of *Botrytis* to this fungicide already exists. Anilazine could be used in a long rotation with other fungicides. Label extension of anilazine for control of *Botrytis* on western hemlock, Douglas-fir, and western redcedar is desirable.

Dichloran (rank 5):

Dichloran gave excellent control of *Botrytis* on western hemlock, very good control on Sitka spruce, and adequate control on the remaining tree species. Growth inhibition of western hemlock, Sitka spruce, and western redcedar would be offset by improved disease control. The *in vitro* tests suggest that no tolerance to dichloran presently exists in these isolates. Label extension for control of *Botrytis* on western hemlock and Sitka spruce should be pursued, with the other species being included if desired.

Captan (rank 6):

Captan provided good control of *Botrytis* on Douglas-fir and adequate control on western redcedar. Control on the other species was adequate to poor. Growth of western hemlock, Sitka spruce, and western redcedar seedlings was inhibited by this fungicide. Tolerance to captan was evident in the *in vitro* tests, particularly at the lower dosages. As captan is already used extensively for the control of *Botrytis* on all tested tree species, label extension should be pursued for this fungicide.

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APPENDIX 1. Sources of Botrytis isolates used as inoculum

Isolate	Isolated from	Nursery	Tolerant to
B1	Douglas-fir	Campbell River	benomyl, chlorothalonil
B2	interior spruce	Koksilah	none
B4	western redcedar	Campbell River	benomyl
B11	Douglas-fir	Surrey	captan, chlorothalonil
B24	interior spruce	Balco	benomyl, chlorothalonil
B27	Douglas-fir	Reid Collins	benomyl, chlorothalonil
B29	western larch	Vernon	benomyl, chlorothalonil
B30	Engelmann spruce	Green Timbers	chlorothalonil
B31	western hemlock	Koksilah	captan, chlorothalonil
B32	Douglas-fir	Surrey	benomyl, captan, chlorothalonil

APPENDIX 2. Average efficacy rankings of the fungicides against gray mould^a

Treatment	Dosage	Tree species				
		Douglas-fir	Western hemlock	Sitka spruce	White spruce	Western redcedar
Control	Inoc	15.5	20.0	17.0	18.0	19.0
	Uninoc	20.0	20.0	20.0	20.0	20.0
Overall rank		7.0	7.0	7.0	7.0	7.0
Anilazine	0.5 X	14.5	8.0	15.5	6.0	8.5
-	1.0X	14.5	5.5	7.0	8.0	8.5
-	2.0X	14.5	11.5	3.0	6.0	8.5
Overall rank		5.5	3.0	3.0	2.0	3.0
Captan	0.5 X	8.0	16.5	15.5	16.5	16.0
-	1.0X	14.5	14.0	15.5	16.5	5.5
-	2.0X	14.5	11.5	10.0	11.5	13.0
Overall rank		4.0	5.0	5.0	6.0	5.0
Dichloran	0.5 X	14.5	8.0	15.5	11.5	17.5
-	1.0X	14.5	5.5	15.5	16.5	17.5
-	2.0X	14.5	8.0	15.5	11.5	15.0
Overall rank		5.5	2.0	6.0	5.0	6.0
Folpet	0.5 X	3.0	18.5	5.0	4.0	8.5
-	1.0X	6.0	14.0	7.0	9.0	13.0
-	2.0X	4.5	14.0	7.0	16.5	11.0
Overall rank		2.0	6.0	2.0	3.0	4.0
Iprodione	0.5 X	4.5	2.5	3.0	2.5	3.0
-	1.0X	2.0	1.0	3.0	2.5	3.0
-	2.0X	1.0	2.5	1.0	1.0	1.0
Overall rank		1.0	1.0	1.0	1.0	1.0
Thiram	0.5 X	8.0	10.0	15.5	16.5	3.0
-	1.0X	14.5	16.5	10.0	6.0	13.0
-	2.0X	8.0	4.0	10.0	11.5	5.5
Overall rank		3.0	4.0	4.0	4.0	2.0
Significant difference ^b		19.0	16.0	19.0	15.5	19.0

^a The lower the ranking, the less severe the disease. "Control Inoc" was inoculated with *Botrytis*; "Control Uninoc" was uninoculated. All controls were treated with tap water. Significant differences were identified using a pairwise comparison test with the probability of a type I error among all comparisons being 10%, while the probability of a type I error in an individual comparison is 0.05%.

^b Reading down, means within a column are significantly different from one another if the difference between the two is greater than the significant difference for that column.

APPENDIX 3. Average severity rankings of gray mould on seedlings treated with the various fungicides^a

Treatment	Dosage	Tree species				
		Douglas-fir	Western hemlock	Sitka spruce	White spruce	Western redcedar
Control	Inoc	18.0	18.5	16.0	18.0	18.0
	Uninoc	20.0	20.0	20.0	20.0	20.0
Overall rank		7.0	7.0	7.0	7.0	7.0
Anilazine	0.5 X	3.5	9.0	13.0	12.5	14.0
	1.0X	18.0	7.0	4.5	6.0	5.5
	2.0X	14.0	8.0	1.0	10.0	11.0
Overall rank		6.0	5.0	2.0	4.5	5.0
Captan	0.5 X	11.5	15.0	19.0	19.0	15.0
	1.0X	3.5	17.0	15.0	16.0	4.0
	2.0X	7.0	11.0	8.5	17.0	9.5
Overall rank		1.0	6.0	6.0	6.0	4.0
Dichloran	0.5 X	7.0	4.0	2.0	6.0	19.0
	1.0X	11.5	2.0	11.0	12.5	17.0
	2.0X	11.5	5.0	4.5	10.0	12.5
Overall rank		4.0	2.0	1.0	4.5	6.0
Folpet	0.5 X	15.0	18.5	17.0	6.0	16.0
	1.0X	17.0	12.5	12.0	8.0	9.5
	2.0X	1.0	10.0	7.0	14.0	2.0
Overall rank		5.0	4.0	5.0	3.0	3.0
Iprodione	0.5 X	19.0	3.0	14.0	3.5	5.5
	1.0X	3.5	1.0	8.5	10.0	8.0
	2.0X	7.0	6.0	4.5	1.5	7.0
Overall rank		3.0	1.0	3.0	1.0	2.0
Thiram	0.5 X	9.0	12.5	18.0	15.0	2.0
	1.0X	3.5	16.0	10.0	1.5	12.5
	2.0X	11.5	16.0	4.5	3.5	2.0
Overall rank		2.0	5.0	4.0	2.0	1.0
Significant difference ^b		19.0	16.5	18.0	16.5	18.0

^a The lower the ranking, the less severe the disease. "Control inoc" was inoculated with *Botrytis*; "Control Uninoc" was uninoculated. All controls were treated with tap water. Significant differences were identified using a pairwise comparison test with the probability of a type I error among all comparisons being 10%, while the probability of a type I error in an individual comparison is 0.05%.

^b Reading down, means within a column are significantly different from one another if the difference between the two is greater than the significant difference for that column.